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Assessment of activated sludge respiratory inhibition of Bis (2-butoxyethyl) adipate

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ABSTRACT

Effect of Bis (2-butoxyethyl) adipate was studied for it's inhibitory effect on the respiration rate of an activated sludge under *in vitro* conditions. The method followed in the study was that described in the OECD guideline for testing of chemicals 209 (Section-4, adopted 4th April, 1984" Activated sludge, Respiration Inhibition Test"). The EC_{50} (3h) of reference item 3,5-dichlorophenol was determined as 24.61 mg/L. The EC_{50} (3h) of Bis (2-butoxyethyl) adipate was determined as 0.0841 g/L.

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KEYWORDS

Bis (2-butoxyethyl) adipate;
3,5-dichlorophenol;
Active sludge and
inhibition.

INTRODUCTION

The inhibitory effect on the respiration rate of microorganisms from an aerated biological clarification stage can be used as a measure of the toxicity of substances. The group of experts of ecotoxicology of the Organization for Economic Co-operation and Development (OECD) has published a method by which the inhibitory effect can be measured[1,2]. The "Effective concentration 50%" (EC_{50}) is given as the value of measurement of inhibition effect[3,4]. The EC_{50} value is the concentration of a substance or substance mixture at which inhibition of the respiration rate is 50%[5-8]. The method described assesses the effect of a test substance on micro-organisms by measuring the respiration rate under defined conditions in the presence of different concentrations of the test substance[9,10]. The purpose of this method is to provide a rapid screening method whereby substances which may adversely affect aerobic microbial treatment plants can be identified, and to indicate suitable non inhibitory concentrations of test substances to be used in biodegradability tests[11].

Bis (2-butoxyethyl)adipate is a high quality plasticizer for polar elastomers with an emphasis on low temperature applications, without compromising high temperature and volatility aspects. It is based on linear glycol ether chemistry. Compared to standard non-aromatic plasticisers, it has a better compatibility with medium to polar plastics, especially at low temperature and in contact with organic solvents and oils. Bis (2-butoxyethyl) adipate is part of a family of new plasticisers which can help in replacing phthalates in current applications. It can be used in nitrile, epichlorohydrine, polychloroprene or other fluorinated rubbers.

EXPERIMENTAL

Materials and methods

Reference standard of Bis (2-butoxyethyl)adipate, 3,5 dichlorophenol were obtained from Sigma Aldrich.

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Peptone and Meat extract were purchased from the Himedia, Bombay. Urea, Sodium chloride, Calcium chloride, Magnesium sulphate, Dipotassium Hydrogen phosphate, Sodium hydroxide, Sulphuric acid AR Grade supplied by Merck Limited.

Preparation of microbial inoculums

The sludge was collected from the Nijam Sugar Mill, Bobbili, Vizianagaram District, Andhrapradesh. The dry weight of the sludge was determined and it was enriched by adding synthetic waste-water (50 mL per litre of synthetic sewage sludge). The mixture was aerated overnight at 20 ± 2°C and used for the study. For measurement of the sensitivity of sludge, the EC_{50} of the standard inhibitor substance 3,5-dichlorophenol was determined after a contact time of three hours.

TEST METHODS

General procedure

Cleaning and sterilization of glassware

All glassware were washed in cleaning solution and rinsed thoroughly in tap water. The cleaned glassware were sterilized by heating at about 100°C for 2 hours in hot air oven.

Measurement of temperature

The temperature of the test solution was recorded using calibrated thermometer.

Preparation of synthetic sewage feed

The following ingredients were dissolved in one liter of water.

Ingredients	Quantity (g)
Peptone	16.2
Meat extract	11.1
Urea	3.2
Sodium chloride (NaCl)	0.7
Calcium chloride ($CaCl_2$. $2H_2O$)	0.4
Magnesium sulphate ($MgSO_4$. $7H_2O$)	0.3
Dipotassium Hydrogen phosphate (K_2HPO_4)	2.9

Preparation of reference substance concentrations

Five hundred milligrams of 3,5-dichlorophenol was dissolved in 10 mL of 1N NaOH and diluted to 30 mL with distilled water. Then 8 mL of 1N H_2SO_4 was added by continuous stirring and finally the volume was made up to one liter with distilled water. The pH of the stock solution was 7.03.

Bottle No.	Treatments	Concentrations (mg/L)
1	Control 1	C1
2		5
3		10
4	3,5-dichlorophenol	20
5		40
6		80
7	Control 2	C2

Preparation of the test substance concentrations

The following concentrations of test item were weighed directly in reaction bottle for the analysis.

Bottle No.	Treatments	Concentrations (g/L)
8	Bis(2-butoxyethyl)adipate	0.1002
9		0.2502
10		0.5002
11		1.025
12		2.012
13	Control 3	C3

TEST PROCEDURE

The following were added into 500 mL flat bottom flask and aerated for 3 h. 16 mL synthetic sewage feed X mL (5,10,20,40 and 80 mL) stock solution of 3, 5–dichlorophenol /x g (0.1002,0.2502, 0.5002, 1.025 and 2.012g) of Bis (2-butoxyethyl)adipate (water in case of controls) 200 mL aerated sewage sludge. Approximately 300 mL water and made up to 500 mL using water. After the end of 3h, the aeration was stopped. The decrease in oxygen was determined over a period of 10 minutes by reading off the oxygen concentration at every minute. The measurements of the oxygen concentrations were carried out with calibrated dissolved oxygen meter.

Calculation

The respiration rate was determined from the linear range of the oxygen decrease. From the rates of oxygen

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decrease, the inhibition of the respiration rate of the sewage sludge was calculated by using the following formula:

$$\text{Percent inhibition} = \left\{ 1 - \left[\frac{2R_s}{(R_{c1}+R_{c2})} \right] \times 100 \right\}$$

Where,

R_s = Oxygen-consumption rate at tested concentration of test substance/ Referenceitem

Rc1 = Oxygen-consumption rate, Control 1

Rc2 = Oxygen-consumption rate, Control 2

Validity criteria

1 The respiration rates of two controls were within in the range (15%) of each other.

2 EC_{50} value for the reference standard 3, 5 dichlorophenol was determined as 26.88 mg/L.

3 The five test concentrations spaced by constant factors within the range 2 – 2.5 was used This value assured the regulatory requirement.

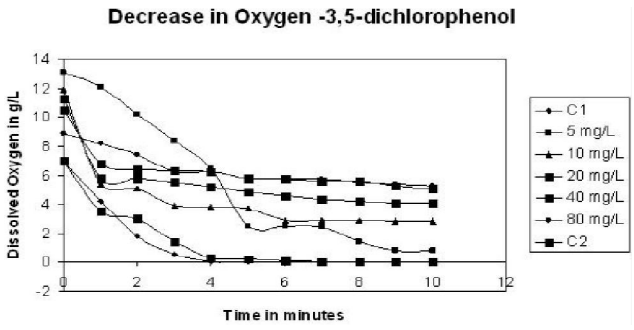

Figure 1 : Decrease in oxygen (3,5-dichlorophenol) over a period of ten minutes

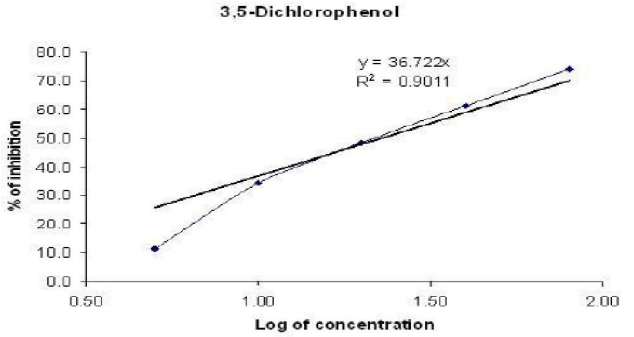

Figure 2 : Inhibition of the respiration rate (3,5-dichlorophenol)

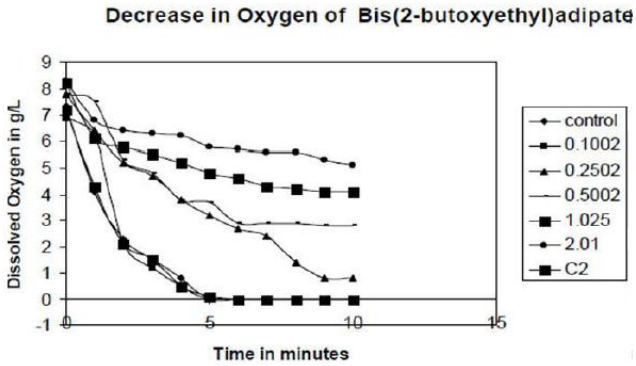

Figure 3 : Decrease in oxygen of bis (butoxyethyl) adipate over a period of ten minutes

TABLE 1 : Determination of The EC_{50} of 3, 5-Dichlorophenol and Bis(butoxyethyl) adipate

Reference Item							Test Item					
3,5-dichlorophenol (mg/l)							Proviplast 0142 (g/L)					
C1	5	10	20	40	80	C2	0.1002	0.2502	0.5002	1.025	2.012	C3
6.9	13.1	11.9	11.3	10.5	8.9	7	7.3	6.9	7.8	7.8	8.2	8.3
4.2	12.1	5.4	5.8	6.8	8.2	3.5	4.1	6.2	6.4	7.5	6.1	6.8
1.8	10.2	5.1	5.8	6.4	7.4	3	2.3	5	5.2	5.3	5.8	6.4
0.5	8.4	3.9	5.5	6.3	6.2	1.4	1.5	4.2	4.7	4.8	5.5	6.3
0	6.5	3.8	5.2	6.2	6.2	0.3	0.8	3.5	3.8	3.8	5.2	6.2
0	2.5	3.7	4.8	5.8	5.8	0.2	0	3.2	3.2	3.7	4.8	5.8
0	2.5	2.9	4.6	5.7	5.8	0.1	0	2.6	2.7	2.9	4.6	5.7
0	2.4	2.9	4.3	5.6	5.7	0	0	2.1	2.4	2.9	4.3	5.6
0	1.4	2.9	4.2	5.6	5.5	0	0	1.2	1.4	2.9	4.2	5.6
0	0.8	2.8	4.1	5.3	5.4	0	0	0.6	0.8	2.8	4.1	5.3
0	0.8	2.8	4.1	5.1	5.3	0	0	0.6	0.8	2.8	4.1	5.1
41.4	73.8	54.6	43.2	32.4	21.6	42	43.8	37.8	42	30	24.6	19.2
50.36	11.5	34.5	48.2	61.2	74.1	49.64	49.66	56.6	51.7	65.5	71.7	77.93
7.73	7.84	7.75	7.68	7.64	7.57	7.76	7.76	7.68	7.54	7.61	7.64	7.75

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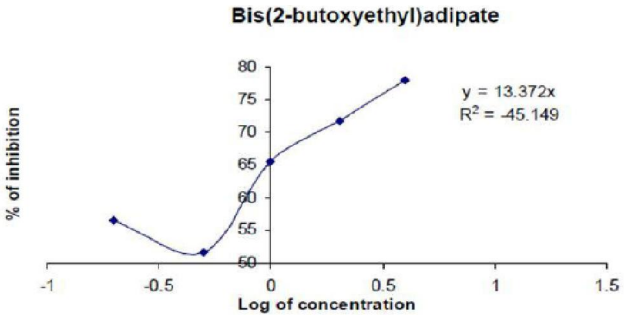

Figure 4 : Inhibition of the respiration rate of bis (butoxyethyl) adipate

RESULTS AND DISCUSSION

The sludge contained 3.2 g/L (dry weight) suspended solids, filterable components. The decrease in oxygen concentration in the test preparation was linear over a wide range. Figure 1 shows the decrease in oxygen concentration after 3h exposure with reference standard 3, 5-dichlorophenol. The respiration rate was determined from the decrease in oxygen. Figure 2 shows the inhibition of the respiration rate against the concentration of 3,5-dichlorophenol. The EC_{50} of 3, 5 – dichlorophenol after an exposure time of three hours was determined as 26.6092 mg/L. Figure 3 shows the decrease in oxygen concentration after 3 h exposure time of Bis (2-butoxyethyl)adipate. Figure 4 shows the inhibition of the respiration rate against the concentration of Bis (2-butoxyethyl)adipate. The results are presented in TABLE 1. The EC_{50} after an exposure time of three hours was determined as 0.0841 g/L.

CONCLUSION

From the results it was observed that the test item is inhibitory to the microbial organisms. The EC_{50} for Bis (2-butoxyethyl)adipate after an exposure time of three hours was 0.0841 g/L.

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